

What is claimed is:

1. Isolated nucleic acid comprising a nucleic acid having at least a 65% sequence identity to (a) a nucleic acid molecule encoding a GFR α 3 polypeptide comprising the sequence of amino acids 27 to 400 of SEQ ID NO: 15 or the sequence of amino acids 27 to 369 of SEQ ID NO: 17, or (b) the complement of the nucleic acid molecule of (a).
2. The isolated nucleic acid of claim 1 comprising a nucleic acid having at least a 65% sequence identity to (a) a nucleic acid molecule encoding a GFR α 3 polypeptide comprising the sequence of amino acids 1 to 400 of SEQ ID NO: 15 or the sequence of amino acids 1 to 379 of SEQ ID NO: 17, or (b) the complement of the nucleic acid molecule of (a).
3. The isolated nucleic acid of claim 1, wherein the GPI anchor sequence is absent or substituted and inactive.
4. The nucleic acid of claim 1, wherein the nucleic acid has at least 75% sequence identity to (a) a nucleic acid molecule encoding a GFR α 3 polypeptide comprising the sequence of amino acids 27 to 400 of SEQ ID NO: 15 or the sequence of amino acids 27 to 369 of SEQ ID NO: 17, or (b) the complement of the nucleic acid molecule of (a).
5. The isolated nucleic acid of claim 1, comprising a nucleic acid encoding a GFR α 3 polypeptide having amino acid residues 27 to 400 of SEQ ID NO: 15 or 27 to 369 of SEQ ID NO: 17.
6. The isolated nucleic acid of claim 1 comprising DNA encoding a GFR α 3 polypeptide having amino acid residues 1 to 400 of SEQ ID NO: 15 or residues 1 to 369 of SEQ ID NO: 17.
7. An isolated nucleic acid comprising nucleic acid having at least a 65% sequence identity to (a) a nucleic acid molecule encoding the same mature polypeptide encoded by the cDNA in ATCC Deposit No. 209752 (designation: DNA48613-1268) or in ATCC Deposit No. 209751, or (b) the complement of the DNA molecule of (a) or (b).
8. The isolated nucleic acid of claim 7, comprising the GFR α 3 encoding sequence of the cDNA in ATCC deposit No. 209752 (designation: DNA48613-1268), in ATCC Deposit No. 209571, or a sequence which hybridizes thereto under stringent conditions.
9. An isolated nucleic acid comprising a nucleic acid having at least a 65% sequence identity to (a) a nucleic acid molecule encoding a GFR α 3 polypeptide comprising the sequence of amino acids 84 to 360 of

SEQ ID NO: 15, amino acids 84 to 329 of SEQ ID NO: 17, or the sequence of amino acids 110 to 386 of SEQ ID NO: 20, or (b) the complement of the nucleic acid molecule of (a).

10. The isolated nucleic acid of claim 9, comprising a GFR α 3 encoding sequence which hybridizes under stringent conditions to (a) a nucleic acid molecule encoding a GFR α 3 polypeptide comprising the sequence of amino acids 84 to 360 of SEQ ID NO: 15, amino acids 84 to 329 of SEQ ID NO: 17, or the sequence of amino acids 110 to 386 of SEQ ID NO: 20, or (b) the complement of the nucleic acid molecule of (a).

11. A vector comprising the nucleic acid of claim 1.

12. The vector of claim 9 operably linked to control sequences recognized by a host cell transformed with the vector.

10 13. A host cell comprising the vector of claim 10.

14. The host cell of claim 11 wherein said cell is a CHO cell, an *E. coli.*, or a yeast cell.

15. A process for producing GFR α 3 polypeptides comprising culturing the host cell of claim 11 under conditions suitable for expression of GFR α 3 and recovering GFR α 3 from the cell culture.

15 16. A polypeptide comprising a sequence having at least 65% sequence identity with amino acid residues 84 to 360 of SEQ ID NO: 15 or 84 to 329 of SEQ ID NO: 17.

17. The polypeptide of claim 16 that is an isolated native sequence GFR α 3 polypeptide.

18. The polypeptide of claim 16 with its GPI anchor sequence absent or substituted and inactive.

19. The polypeptide of claim 16 comprising amino acid residues 27 to 400 of SEQ ID NO: 15, amino acid residues 27 to 369 of SEQ ID NO: 17, amino acid residues 84 to 360 of SEQ ID NO: 15, or amino acid residues 110 to 386 of SEQ ID NO: 20.

20. A chimeric molecule comprising a GFR α 3 polypeptide fused to a heterologous amino acid sequence.

21. The chimeric molecule of claim 20 wherein said heterologous amino acid sequence is an epitope tag sequence.

22. The chimeric molecule of claim 20 wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

23. An antibody which specifically binds to GFR α 3 polypeptide.

24. The antibody of claim 23 that is an agonist antibody.

25. The use of the antibody of claim 23 to treat a neuronal disorder of the periphery.

26. A method for measuring agonist binding to a polypeptide comprising an agonist-binding domain of an α -subunit receptor, comprising the steps of exposing the polypeptide positioned in a cell membrane to a candidate agonist and measuring homo-dimerization or homo-oligomerization of the polypeptide.

27. The method of claim 26, wherein the α -subunit receptor is a GFR α -receptor.

28. The method of claim 26, wherein the polypeptide further comprises a dimerization- or oligomerization-activated enzymatic domain and homo-dimerization or homo-oligomerization is detected by measuring enzymatic activity of the polypeptide.

29. The method of claim 28, wherein the enzymatic domain is the intracellular autocatalytic domain of a receptor tyrosine kinase and homo-dimerization or homo-oligomerization is detected by measuring autophosphorylation of the polypeptide.

30. A method of measuring autophosphorylation of a polypeptide receptor construct comprising a ligand-binding domain of an α -subunit receptor, the intracellular catalytic domain of a tyrosine kinase receptor, and a flag epitope, comprising the steps of:

- (a) coating a first solid phase with a homogeneous population of eukaryotic cells so that the cells adhere to the first solid phase, wherein, positioned in their membranes, the cells have the polypeptide receptor construct;
- (b) exposing the adhering cells to an analyte;
- (c) solubilizing the adhering cells, thereby releasing cell lysate therefrom;
- (d) coating a second solid phase with a capture agent which binds specifically to the flag epitope so that the capture agent adheres to the second solid phase;
- (e) exposing the adhering capture agent to the cell lysate obtained in step (c) so that the receptor construct adheres to the second solid phase;
- (f) washing the second solid phase so as to remove unbound cell lysate;
- (g) exposing the adhering receptor construct to an anti-phosphotyrosine antibody which identifies phosphorylated tyrosine residues in the tyrosine kinase receptor; and
- (h) measuring binding of the anti-phosphotyrosine antibody to the adhering receptor construct.

31. The method of claim 30 wherein the cells are transformed with nucleic acid encoding the receptor construct prior to step (a).
32. The method of claim 30 wherein the cells comprise a mammalian cell line.
33. The method of claim 30 wherein the cells are adherent.
- 5 34. The method of claim 30 wherein the capture agent comprises a capture antibody.
35. The method of claim 30 wherein the first solid phase comprises a well of a first assay plate.
36. The method of claim 30 wherein the anti-phosphotyrosine antibody is labelled.
37. The method of claim 36 wherein the label comprises an enzyme which is exposed to a color reagent and the color change of the color reagent is determined in step (h).
- 10 38. The method of claim 30 wherein the flag polypeptide is fused to the amino terminus of the α -subunit receptor ligand-binding domain.
39. The method of claim 30 wherein the flag polypeptide is fused to the carboxyl terminus of the tyrosine kinase receptor intracellular catalytic domain.
40. The method of claim 30 wherein the tyrosine kinase receptor is a Rse receptor, a trk A receptor, a trk B receptor or a trk C receptor.
- 15 41. The method of claim 30 wherein the α -subunit receptor is a GFR α -receptor.
42. The method of claim 40 wherein the receptor construct further comprises the transmembrane domain of the Rse receptor and the flag epitope comprises the GD polypeptide.
43. The method of claim 30 wherein the analyte comprises an agonist for the α -subunit receptor.
- 20 44. The method of claim 30 wherein the analyte comprises an antagonist for the α -subunit receptor.
45. The method of claim 44 wherein the antagonist competitively inhibits binding or activation of the α -subunit receptor by an agonist thereto and step (b) is followed by a step wherein the adhering cells are exposed to the agonist.

46. The method of claim 30 wherein the analyte is a composition which comprises an antagonist and an agonist for the α -subunit receptor and the assay measures the ability of the antagonist to bind to the agonist and thereby reduce activation of the polypeptide construct by the agonist.

47. A method for measuring autophosphorylation of a polypeptide receptor construct comprising a ligand-binding domain of an α -subunit receptor, the intracellular catalytic domain of a tyrosine kinase receptor, and a flag epitope, comprising the steps of:

- (a) coating a well of a first assay plate with a homogeneous population of adherent cells so that the cells adhere to the well, wherein the cells have the polypeptide receptor construct positioned in the cell membranes thereof;
- (b) exposing the adhering cells to an analyte;
- (c) solubilizing the adhering cells thereby releasing cell lysate therefrom;
- (d) coating a well of a second assay plate with a capture agent which binds specifically to the polypeptide receptor construct so that the capture agent adheres to the well;
- (e) exposing the cell lysate obtained in step (c) to the adhering capture agent so that the polypeptide receptor construct adheres to the well;
- (f) washing the well so as to remove unbound cell lysate;
- (g) exposing the adhering polypeptide receptor construct to an anti-phosphotyrosine antibody which binds selectively to phosphorylated tyrosine residues in the polypeptide receptor construct;
- (h) measuring binding of the anti-phosphotyrosine antibody to the adhering polypeptide receptor construct.

48. The method of claim 47 wherein the α -subunit receptor is a GFR α -receptor.

49. A polypeptide comprising an α -subunit receptor ligand-binding domain, a flag polypeptide, and an intracellular catalytic domain of a tyrosine kinase receptor.

50. The polypeptide of claim 49, wherein the flag polypeptide comprises the gD flag epitope.

51. The polypeptide of claim 49, wherein the tyrosine kinase receptor is a Rse receptor.

52. The polypeptide of claim 51 further comprising the transmembrane domain of the Rse receptor.

54. The polypeptide of claim 53, wherein the α -subunit receptor is a GFR α receptor.

55
56. A kit comprising a solid phase coated with a capture agent which binds specifically to a flag polypeptide, and a polypeptide comprising an α -subunit receptor ligand-binding domain, a flag polypeptide, and an intracellular catalytic domain of a tyrosine kinase receptor.

56
57. The kit of claim 56 wherein the solid phase comprises a well of a microtiter plate.

57
58. The kit of claim 56 further comprising a labeled anti-phosphotyrosine antibody.

58
59. The kit of claim 58 wherein the label comprises an enzyme.

59
60. The kit of claim 56 further comprising a cell transformed with a nucleic acid encoding a polypeptide comprising an α -subunit receptor ligand-binding domain, a flag polypeptide, and an intracellular catalytic domain of a tyrosine kinase receptor.

60
61. An assay for measuring phosphorylation of polypeptide receptor construct comprising a ligand-binding domain of an α -subunit receptor, the intracellular catalytic domain of a kinase receptor, and a flag epitope, comprising the steps of:

- (a) coating a first solid phase with a homogeneous population of eukaryotic cells so that the cells adhere to the first solid phase, wherein the cells comprise the polypeptide receptor construct;
- (b) exposing the adhering cells to an analyte;
- (c) solubilizing the adhering cells, thereby releasing cell lysate therefrom;
- (d) coating a second solid phase with a capture agent which binds specifically to the flag polypeptide so that the capture agent adheres to the second solid phase;
- (e) exposing the adhering capture agent to the cell lysate obtained in step (c) so that the receptor construct adheres to the second solid phase;
- (f) washing the second solid phase so as to remove unbound cell lysate;
- (g) exposing the adhering kinase construct to an antibody which identifies phosphorylated residues in the receptor construct; and
- (h) measuring binding of the antibody to the adhering receptor construct.

61
62. The assay of claim 61 wherein the α -receptor is a GFR α -receptor.

62
63. The assay of claim 61 wherein the kinase receptor is a serine-threonine kinase receptor.

63
64. The assay of claim 61 which measures phosphatase activity.

Rule 1.126
Revised
6/14/00

64
65.

65
66.

The assay of claim 64 wherein the cells further comprise a phosphatase and the assay further comprises the step of exposing the eukaryotic cells to a phosphatase inhibitor prior to step (c).

The assay of claim 64 which further comprises the steps in between steps (f) and (g) of exposing the adhering kinase construct to a phosphatase and then washing the second solid phase so as to remove unbound phosphatase.

5

AdH
Hi

AdH
Hi

AdH
Hi

666120" 5232260